

AAT is an innovative company and owns its unique technology.

As AAT main goal is to provide microarrays for researchers, clinical industries as everyday genomic tools, we have developed and patented process:

- functionalisation of the slides,
- design of capture probe for hybridization of homologous sequences,
- the colorimetric labeling,
- the detector,
- the DNA sequences

AAT's experience in the field of microarrays can help you to develop your. Please click here to get more information on AAT customized microarrays.

You can find here a short list of AAT patents related to the chips technology.

### AAT introduces the Bio-CD™

Combining numerical and biological information, this new standard in microarrays is the perfect tool for diagnosis.

Containing up to 20 microarrays per CD, additional information is included and recorded on the numerical track: patient data, microarray data...

Click here to get more information about AAT Bio-CD™

(c) Advanced Array Technology S.A. 2002

[webmaster@aat-array.com](mailto:webmaster@aat-array.com)

BEST AVAILABLE COPY

APPENDIX 1

Advanced Array Technology - Rat HepatoChips

# Rat HepatoChips: A DNA microarray for drug side-effect detection

APPENDIX 2

## Rat HepatoChips - Table of contents

Why low-density DNA microarrays?	2
Microarrays applied to gene expression principle	4
Rat HepatoChips challenge	5
Rat HepatoChips principle	6
List of the genes used on the Rat HepatoChips	7
Scanning, quantification & data processing	8
Rat HepatoChips results	9
Overview of Rat HepatoChips development	11
Rat HepatoChips validation	12
Validation results	13
Rat HepatoChips kit and service	14
Upcoming challenges	15
Other AAT products	16
Contact AAT	17

## Rat HepatoChips Why low-density DNA microarrays?

Now that:

- More than 24 entire genomes are sequenced
- More than 90% of the coding sequences of the human genome are known
- More than 100 sequencing projects are in progress
- More than 3 billion DNA bases are available in the data banks

To use this huge amount of data, tools need to be developed: **microarrays** are the bottleneck for **easy detection of genes and better understanding of their role & function** in pathologies.

The greatest aim of DNA microarrays is to offer **new technologies** usable to streamline **drug development**, completing existing procedures with the following advantages:

- **Speed:** the same experiments can be performed in significantly less time.
- **Simultaneous experimentation:** scientists can screen hundreds of genes in a single experiment.
- **Cost:** reducing the assays for drug screening implies shortening of drug development cost

## Rat HepatoChips

### Why using low-density DNA microarrays?

The products developed by Advanced Array Technology are low-density microarrays, that means from 10 to 400 spots per array. The choice of this technology is explained by:

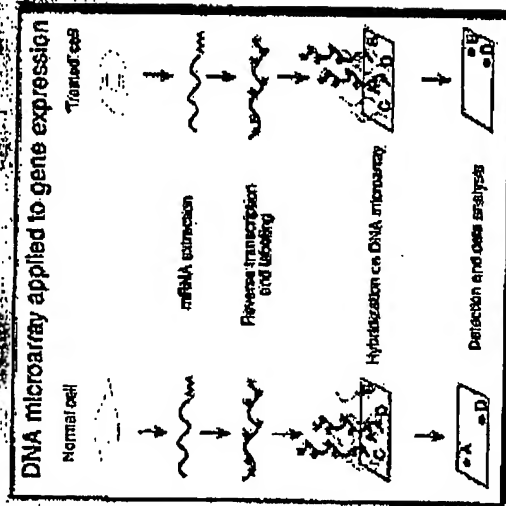
- The possibility to optimize each capture probe to get comparable hybridization yield and to allow reliable quantification
- The easier and more efficient data processing and mining
- The lower cost per test

Advanced Array Technology provides pharmaceutical industries comprehensible, user-friendly & ready-to-use tools for molecular toxicology.

## Rat HepatoChips

### Microarrays applied to gene expression: principle

The microarray surface carries highly specific DNA probes. After mRNA extraction, reverse-transcription and labeling with biotin, the hybridization on the DNA microarrays is performed. Fluorescence readout allows detection of the genes expressed in the cells.



Analysis is performed by quantification of the cellular mRNA: comparison between reference cells and treated cells allows analyzing differentially expressed genes in response to a chemical or environmental stress.

## Rat HepatoChips: Challenge

AAT Rat HepatoChips is a perfect tool specially designed for pharmaceutical industries and research laboratories.

The use of Rat HepatoChips answers the following key questions:

- Screening and identification of chemical compounds with potential side effects
- Gives information on a number of toxicology issues, including mode of action, dose-response relationships and chemical interactions
- Allows to discard toxic compounds early in the discovery process of new drugs
  - Time, working and money savings
- Better understanding of the toxicity mechanism
- Reducing animal use (ethic and industrial objective)

## Rat HepatoChips Challenge

HepatoChips is a quantitative microarray specially designed for gene expression analysis of the main rat cellular functions.

It gives a view of how a chemical compound, a new synthesized drug or a transfected gene will affect gene expression involved in the following cellular functions:

- apoptosis,
- DNA repair,
- general & xenobiotic metabolism,
- stress response,
- transcription & growth factors,
- cell cycle,
- inflammation,
- oncogene,
- peroxisome proliferators,
- Transport.



# Rat HepatoChips

## List of the genes used on the Rat HepatoChips

59 genes of rat have been carefully selected by experts in toxicology to identify the potential side-effect of compounds.

Gene Categories	Selected genes
Apoptosis	Bax, Bcl-2, TNF, Smp30
Cell cycle	Cyclin D1, JNK-1, Telomerase
DNA damage/Repair	GADD45, GADD153, MGMT
Inflammation	IL-6, cyclooxygenase-2
Metabolism	P450s, glutathione enzymes, glucoronidation enzymes
Oncogene	c-jun, c-myc, elk-1
Stress response	Oxidative stress genes, ApoJ, Hsp70, Heme oxygenase 2, SOD
Peroxisome Proliferators	Enoyl coA hydratase, PPAR $\alpha$ , Acyl coA oxidase
Transcription factors, growth factors	C/EBP $\beta$ , I $\kappa$ B- $\alpha$ , NF $\kappa$ B, erk-1, p38, HGF, TGF $\beta$ RII
Transport	Multi-drug Resistance protein, albumin, transferring

## Rat HepatoChips Scanning, quantification & data processing

The hybridized microarrays were scanned with a confocal scanner (fluorescence readout) and quantified with the software Imagen<sup>TM</sup> of BioDiscovery, Inc.

A software for data processing and statistical analysis has been developed to treat raw data on an MS-Excel<sup>TM</sup> template. The data are normalized by a two steps correction taking based on:

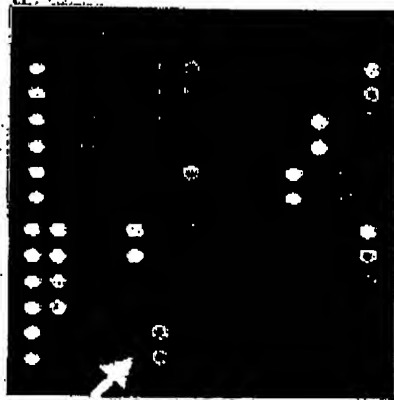
- three internal standards (from genes of plants) spiked into the reverse-transcription in different amounts. These internal standards are spotted on different areas of the microarray, in order to make allowance for the background inhomogeneity.
- eight housekeeping genes included on the microarray.

The ratios between gene expression in normal cells and treated cells are determined as significant by a statistical test.

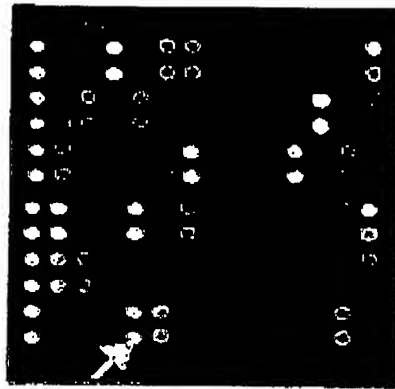
## Rat HepatoChips Results

Here is an example of the results obtained after scanning a Rat HepatoChips microarray.

The arrows show the differentially expressed genes between reference cells and treated cells. All spots are in duplicate for reliability reasons.



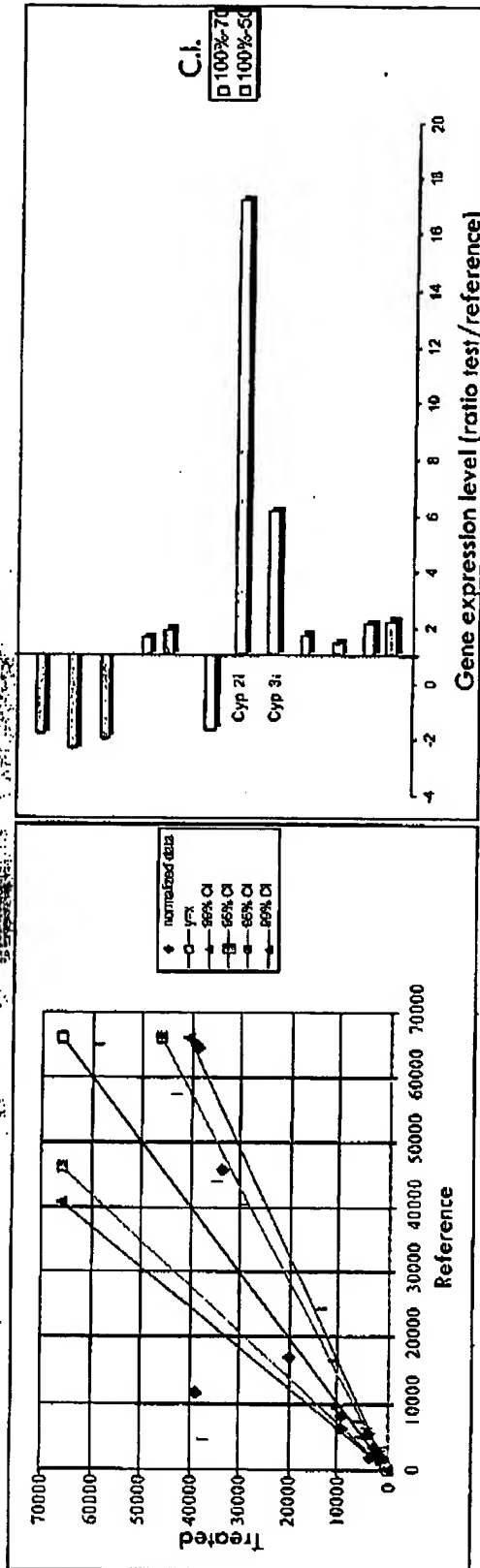
Reference



Test

# Rat HepatoChips Results

Further analysis can be done from the quantitative results



## Rat HepatoChips

### Overview of Rat HepatoChips development

In order to be reliable and easy to use, Rat HepatoChips development process has required optimization of all the steps including:

- DNA capture probe synthesis
- Covalent attachment of capture probe on glass slides
- Specificity of capture probe
- Optimization of all reverse transcription and hybridization conditions
- Hybridization of cDNA from non treated and treated hepatocytes with toxic reference compounds
- Data processing and data mining

Pharmaceutical companies and research laboratories have validated rat HepatoChips.

## Rat HepatoChips Validation

Two pharmaceutical companies have validated the Rat HepatoChips to demonstrate its reliability and the reproducibility.

The validation of this gene expression microarray has been completed with nine reference compounds: PB, PCN, Dex, ISN, MIC, TAO, BNF, MCP, CLOT.

The aim was to check if the corresponding cytochrome P450s were induced, to compare the method with in vitro assay results and in vivo traditional techniques.

Reproducibility has been estimated by variation between experiments and animal-to-animal variation.

## Rat HepatoChips Validation Results

The main goal was to validate the Rat HepatoChips as an efficient and reliable tool for studying changes in gene expression due to drug treatment.

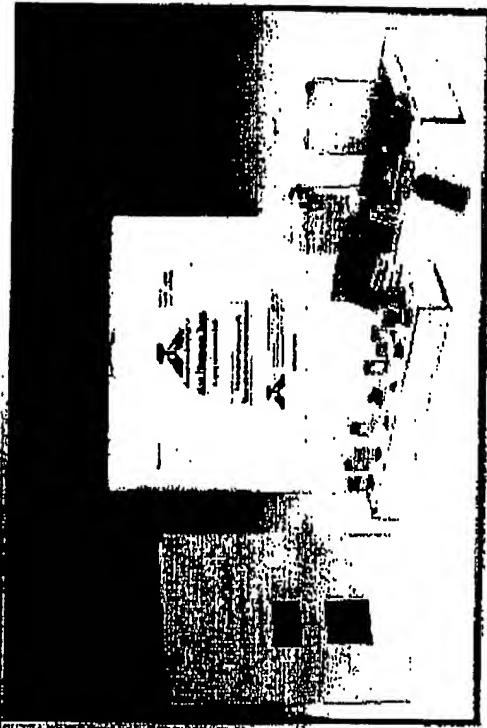
The results showed excellent:

- Reliability: the corresponding P450s were induced due to the treatment with the several reference compounds
- Reproducibility: very low levels of variability reached:
  - spot-to-spot = 6%
  - array-to-array (from a same batch) = 10%
  - array-to-array (from different batch) = 12%



## Rat HepatoChips Kit & Service

- AAT Rat HepatoChips Kit includes:
- 5 slides (2 arrays/slide)
  - Internal standards & positive controls
  - Hybridization and detection reagents



AAT also provides a service for the Rat HepatoChips. Just send us your mRNA sample and we do the steps: RT and labeling, hybridization, scanning, quantification and analysis.

Please feel free to contact us to get more information about this service



## Upcoming challenges

Due to the fast development of genomic and proteomic technologies, the field is constantly evolving and growing. AAT is developing increasingly complex microarrays in collaboration with renowned institutes.

Main projects in development in AAT research laboratories

Name	Applications
TumoChip	Tools for better medical treatment of cancer
ABC Chip	Adaptation of treatment in cancer patients
CNS Chip: central nervous systems including G protein coupled receptor	Screening of new antagonists or antagonists of receptors
Arthero Chip	Monitoring the evolution of arteriosclerosis
LymphoChips	Diagnostic of lymphomas
SeneChips	To follow the ageing process and the stresses
AdipoChips	To follow the differentiation of adipocytes
BioChips	A human general microarray

## Other AAT products

AAT product panel covers the microarray field, from the glass slides to the array detector.

- MagéChips: a gene expression detection microarray
- StaphyChips: a DNA detection microarray
- DiaGlass Slides: new aldehyde activated glass slides
- Silver Blue: a new colorimetric detection method developed for microarrays
- WorkStation: a new colorimetric detector

More information about AAT products can be found on our website:  
[www.aat-array.com](http://www.aat-array.com)

## Contact AAT

- Web addresses:

Website: [www.aat-array.com](http://www.aat-array.com)

General & Commercial information: [aatinfo@aat-array.com](mailto:aatinfo@aat-array.com)

Production & technical information: [aattech@aat-array.com](mailto:aattech@aat-array.com)

- Mailing address:

Advanced Array Technology S.A.

Rue du Séminaire, 22

5000 NAMUR

BELGIUM

tel: +32-81-72-41-51

fax: +32-81-72-51-28

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**